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RESEARCH ARTICLE

Evaluation of Extended Spectrum of Beta Lactamases Producing Escherichia coli Isolated From Hospitalized Patients

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Abstract

Multidrug resistances are of utmost importance for the clinical impact of extended spectrum of beta lactamase producing strain of Gram negative bacilli. The present study was used to carry out the detection of extended spectrum of beta lactamase producing *Escherichia coli.* A total of 50 urine samples were collected from various clinically associated patients. Among 50 samples, *Escherichia coli* were found to be predominant (36%). The isolates of *Escherichia coli* were highly resistant to various antibiotics such as Ampicillin, Aztreonam and Cephotaxime. They are sensitive to Ampicillin clavulnate, Cephotaxime clavulnate, Co- trimoxazole, Ertapenem and Imipenem. Extended spectrum of beta lactamase *Escherichia coli* was detected by Double disk synergy test, Modified double disk method and Disk replacement method. The *Escherichia coli* isolates exhibited greater than 5mm zone of inhibition to various antibiotics such as Cephotaxime clavulnate, Cephotaxime alone, Ampicillin clavulnate and Ampicillin alone, which were indicated as positive result for Extended Spectrum of Beta Lactamase production. Plasmid characterization of extended spectrum of Beta lactamases producing strains of *Escherichia coli* was carried out by alkaline lysis method and the plasmid bands were observed in *Escherichia coli* ranging from 10,000bp to 8,000bp. In conclusion, potentially *Escherichia coli* resistance to almost all licensed antibiotics may emerge in the future. Constant and careful worldwide surveillance for multidrug resistance and extended spectrum of beta lactamases producing *Escherichia coli* is urgently warranted.

Introduction

Beta lactam antimicrobial agents are the most commonly used treatment of bacterial infection. Resistance to beta lactam antibiotics among clinical isolates of *Escherichia coli* is most often due to the production of Beta lactamases (Kotra et al., 2002).Extended spectrum of beta lactamases are identified worldwide and have been found in number of different organism particularly in *Escherichia coli* (Goussards and Courvalin, 1999).The extended spectrum of beta lactamases was found to confer resistance to broad spectrum cephalosporin. The promising spectrum of third generation cephalosporin antibiotics used in the treatment of multidrug resistance *Escherichia coli* infection (Abdul and kumar, 2005). The widespread use of antibiotics coupled with the transmissibility of resistance determinants mediated by plasmid. The plasmid that harbor genes encoding extended spectrum beta lactamases factor that contribute to increase in antibiotic resistance in *Escherichia coli* as a bacterial pathogen (Kang et al., 2005).The widespread occurrence of multidrug resistance *Escherichia coli* in the environment has necessitated the need for regular monitoring of antibiotics trends to provide the basis for developing rational prescription programmes and assessing their effectiveness (Idia et al., 2006).

This study evaluated multidrug resistance towards extended spectrum beta lactamases in *Escherichia*

coli isolated from hospitalized patients. In addition to consider the plasmid content of extended spectrum beta lactamases producing *Escherichia coli.*

Material and Methods

2.1. Sample Collection and Handling:

This study was performed at a hospital who admitted hospital in at after 24 hours. Mid stream urine specimens were collected from K.A.P. Vishwanatham government medical college, Tiruchirappalli, Tamil nadu, India. A total of 50 clinical urine samples were collected from hospital admitted patient at study enrollment. All the specimens were processed within 24 hours of collection.

2.2. Identification of Isolates:

Morphological and biochemical analysis were carried out by various microbiologically significant examinations such as Gram staining, Motility, Cultural characteristic of growth and various important biochemical methods were used to identify the growth of the bacteria.

2.3. Disc Diffusion Method:

According to the guidelines of the Clinical Laboratory Standard Institute, Multi drug resistance was detected by using Disk Diffusion test which was performed on Muller- Hinton agar medium. The plates were incubated at 37^0C for 24 hours. Any growth with less than 12 mm in diameter zone around the disk was considered indicative of drug resistance to the bacterial growth.

3. Screening of Extended Spectrum Beta Lactamases

3.1. Double Disk Synergy Test:

As per Clinical and Laboratory Standard Institute (CLSI) guidelines, the combined disk method depends on comparing the zone of inhibition around disks containing an indicator Cephalosporin with and without clavulanic acid. Disk containing Ceftazidime (30mg) alone and Ceftazidime along with an inhibitor Cephalosporin such as Clavulanate (30/10mg) were placed on the inoculated Muller Hinton agar plates.

3.2. Modified Double Disk Diffusion Method:

The standardized test strains were inoculated in Muller-Hinton agar plates. Disk containing expanded-spectrum of Cephalosporins. Cefepime (30mg), Cephotaxime (30mg), Ceftazidime (30mg), Cefpirome (30mg) are placed 30 mm (center to center) from an Amoxicillin-Clavulanate (Ac 30/10mg) disk. After overnight incubation at 37^0C, the detection of an extended spectrum beta lactamases by the test organism is inferred by the presence of characteristic distortion or expansion of the inhibition zones towards the amoxicillin-clavulanate disk

3.3. Disk Replacement Method for Extended Spectrum Beta Lactamases Confirmation:

The standardized test strains were inoculated in Muller-Hinton agar plates**.** Two disk containing Amoxyclav were placed, after 1 hour at room temperature, the disc were removed and replaced with Ceftazidime and Cefuroxime. After overnight incubation at 37^0C, the production of an ESBL by the test organism was detected.

4. Plasmid Analysis:

Plasmids can be isolated by one commonly used technique developed by Birnboim and Dolly involves alkaline lysis. This method relies on bacterial lysis by sodium dodecyl sulphate (SDS) followed by neutralization with a high concentration of low pH potassium acetate. This gives selective precipitation of bacterial chromosomal DNA and other high molecular weight cellular components. The plasmid DNA remains in suspension is precipitated with isopropanol.

Results

The present study was used to carry out the detection of extended spectrum of beta lactamases producing Gram negative *Escherichia coli* strains. A total of 50 urine samples were collected from various clinically associated patients, especially painful urination, pregnancy, Hemodialysis, hospital stay patients, frequent urination and bladder catheter (Table 1).

50 samples were analyzed by microbiological methods. According to the Bergey's manual of determinative bacteriology, the isolates were detected as *Escherichia coli*. Among 50 samples, *Escherichia coli* was found in 18 samples which were identified by various biochemical tests such as Indole, Methyl Red, Nitrate reduction test, Catalase test, TSI agar test and green metallic sheen was produced on EMB agar and lactose fermentation was showed on Mac Conkey agar(Table 2).

In antibiotic disk diffusion method, 33 various concentrations of antibiotics were used against *Escherichia coli* (Table 3). In *Escherichia coli*, Ampicillin (100%), Aztreonam (100%) and Cephotaxime (100%) were highly resistant, followed by Cefixime, Cefoxitin, Ceftazidime, Cefuroxime and Cefpirome were also resistant to *Escherichia coli*. whereas Ampicillin Clavulnate (94.4%), Imipenem (94.4%), Ertapenem (88.8%), Amoxy clavulnate (83.3%) are found highly sensitive in various urine sample (Table 4 & 5).

According to the Clinical and Laboratory Standard Institute published guidelines for performing an Extended Spectrum of Beta lactamase confirmatory test. In *Escherichia coli* isolates produced Extended Spectrum of Beta lactamase activity and it was observed by using various screening test.

In Double disk synergy test, Cephotaxime disk were expanded by the Cephotaxime Clavulnate on *Escherichia coli*. The zone of inhibition was measured as greater than 5mm between two antibiotics such as Cephotaxime and Cephotaxime Clavulnate were detected as Extended Spectrum of Beta Lactamases production.

In Modified Double Disk Diffusion test on *Escherichia coli*, Zone of inhibition around the test disk such as Cefepime, Cephotaxime, Ceftazidime and Cefpirome were increased towards the disk containing Amoxycillin Clavulanic acid as an indicative of extended spectrum beta lactamases production.

In Disk Replacement Method on *Escherichia coli*, two Amoxycillin Clavulnate disk were placed and were removed after 1 hour at room temperature and replaced with Ceftazidime and Cefuroxime. In extended spectrum beta lactamases production, replacement disk produced inhibition zone greater than 5mm which were indicated as positive result for extended spectrum beta lactamases detection.

In Plasmid DNA analysis, the majority of extended spectrum beta lactamases are plasmid mediated and has been applied to the *Escherichia coli* isolates. The *Escherichia coli* strain possesses plasmid ranging from 10,000bp to 8,000base pairs.

Table: 1 Isolation of *E.coli* from various sources of infectious patients

S. No	Age	Sex	Symptoms	Organism
1.	21	F	Pregnancy	*E.coli*
2.	34	M	Foul smelling urine	*E.coli*
3.	67	M	Hemodialysis	*E.coli*
4.	8	F	Urine with strange smell	*E.coli*
5.	25	M	Hospital stay	*E.coli*
6.	45	F	Diabetes	*E.coli*
7.	63	M	Bladder catheter	*E.coli*
8.	59	M	Surgery	*E.coli*
9.	40	F	Hemodialysis	*E.coli*
10.	53	F	Pain during urination	*E.coli*
11.	45	M	Frequent urination	*E.coli*
12.	80	M	Loss of bladder control	*E.coli*
13.	49	F	Bladder catheter	*E.coli*
14.	30	F	Pregnancy	*E.coli*
15.	51	M	Pain during urination	*E.coli*
16.	15	F	Painful urination	*E.coli*
17.	85	F	Pyuria	*E.coli*
18.	39	F	Hospital stay	*E.coli*

Table: 2 Morphological, cultural and biochemical characterization of *E.coli*

S. No	Name of the test	*E.coli*
1.	Gram – staining	Gram negative rod
2.	Motility	Motile
3.	Eosin Methylene blue agar	Green metallic sheen
4.	Mac conkey agar	Lactose fermentation-Red colonies
5.	Indole	Positive
6.	Methyl Red	Positive
7.	Voges Proskauer	Negative
8.	Citrate	Negative
9.	Urease	Negative
10.	Nitrate reduction test	Positive
11.	Catalase	Positive
12.	Oxidase	Negative
13.	ONPG Test	Positive
14.	Triple Sugar Iron agar test	Acid, gas and no H2S production
15.	Casein hydrolysis	No utilization
16.	Starch hydrolysis	No utilization

Table 3: Disk diffusion pattern of E.coli

S. No.	Antimicrobial agent	Conc. (mg)	R	I	S
1.	Amikacin	30	-	9	9
2.	Ampicillin	10	18	-	-
3.	Ampicillin/clav	30	-	1	17
4.	Ampicillin/sulbactam	10/10	1	8	9
5.	Amoxy/clav	30	-	3	15
6.	Aztreonam	30	18	-	-
7.	Cefepime	30	10	5	3
8.	Cefepime/Tazobactam	30/10	-	4	14

9.	Cefixime	5	13	4	1
10.	Cefoxitin	30	12	3	3
11.	Cefpirome	30	11	4	3
12.	Cefpodoxime	10	8	6	4
13.	Ceftazidime	30	12	4	2
14.	Ceftazidime/Tazo	30/10	-	8	10
15.	Ceftizoxime	30	6	8	4
16.	Cefuroxime	30	12	6	-
17.	Cephalothin	30	14	2	2
18.	Cephotaxime	10	18	-	-
19.	Cephotaxime/clav	30	-	6	12
20.	Ciprofloxacin	5	4	7	8
21.	Co Trimoxazole	25	1	5	12
22.	Ertapenem	10	-	2	16
23.	Gentamycin	10	1	4	14
24.	Imipenem	10	-	1	17
25.	Levofloxacin	5	6	2	10
26.	Meropenem	10	-	4	14
27.	Moxifloxacin	5	2	10	6
28.	Nitrofurantoin	300	-	8	10
29.	Norfloxacin	10	9	3	6
30.	Ofloxacin	5	3	6	9
31.	Piper/Tazobactam	100/10	-	7	12
32.	Tetracycline	30	2	2	4
33.	Trimethoprim	25	7	6	5

R – Resistant, I – Intermediate sensitive & S- Sensitive

Table: 4 Resistance pattern of *E.coli*

S. No	Antimicrobial agent	Concentration (mg)	No. of resistance sample	Percentage (%)
1.	Ampicillin	10	18	100
2.	Aztreonam	30	18	100
3.	Cefixime	5	13	72.2
4.	Cefoxitin	30	12	66.6
5.	Cefpirome	30	11	61.1
6.	Ceftazidime	30	12	66.6
7.	Cefuroxime	30	12	66.6
8.	Cephotaxime	10	18	100

Table: 5 Sensitive pattern of *E.coli*

S.No	Antimicrobial agent	Concentration (mg)	No. of sensitive sample	Percentage (%)
1.	Amikacin	30	9	50
2.	Amoxy/clav	30	15	83.3
3.	Ampicillin/clav	30	17	94.4
4.	Ampicillin/sulbactam	10/10	9	50
5.	Cefepime/Tazobactam	30/10	14	77.7
6.	Ceftazidime/Tazobactam	30/10	10	55.5
7.	Cephotaxime/clav	30	12	66.6
8.	Ciprofloxacin	5	8	44.4
9.	Co trimoxazole	25	12	66.6
10.	Ertapenem	10	16	88.8
11.	Imipenem	10	17	94.4
12.	Meropenem	10	14	77.7
13.	Piper/Tazobactam	100/10	12	66.6

Discussion

Extended spectrum beta lactamases are medically importance and patient infected with extended spectrum beta lactamases producing *Escherichia coli* has a greater experience of poor outcome if they are treated with inappropriate antibacterial(Patterson et al.,2001).Clinical failure has been registered because of the emergence of resistance while a patient is uptake Cephamycin of Cefoxitin and Cefotetan therapy. In addition, increasing numbers of Extended spectrum beta lactamases producing *Escherichia coli* express multidrug resistance beta lactamases enzymes that mediate resistance to Cephamycins (Rebuck et al.,2000). Currently, Carbapenems are clinically regarded as the preferred agent for treatment of infectious due to Extended spectrum beta lactamases producing bacteria (Patterson, 2000& Wong-Beringer, 2001).Newer Carbapenems such as Ertapenem also exhibit greater activity against extended spectrum beta lactamases producing bacteria(Yu et al.,2002).The emergence of plasmid mediated extended spectrum beta lactamases among member of *Escherichia coli* has increased worldwide. Plasmid-encoded antibiotic resistance encompasses most **(**Bennett, 2008).Resistance to various antibiotics is relatively common in extended.spectrum.of.beta.lactamases producing *Escherichia coli* strains and it is frequently plasmid mediated.

Conclusion

In conclusion, multidrug resistance of *Escherichia coli* demands a multitalented approach for developing new antibacterial agents is used more effective to control increasing antibiotic resistance measures.

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